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Abstract

Introduction: This study examines the role of nutritional status during exercise training in patients with type 2 diabetes mellitus by investigating the impact of endurance-type exercise training in the fasted versus the fed state on clinical outcome measures, glycemic control, and skeletal muscle characteristics in male type 2 diabetes patients.

Methods: Twenty-five male patients (HbA1c 57±3 mmol/mol (7.4±0.3%)) participated in a randomized 12-week supervised endurance-type exercise intervention, with exercise being performed in an overnight fasted state (FastEx, *n*=13) or after consuming breakfast (FedEx, *n*=12). Patients were evaluated for glycemic control, blood lipid profiles, body composition and physical fitness, and skeletal muscle gene expression.

Results: Exercise training was well tolerated without any incident of hypoglycemia. Exercise training significantly decreased whole-body fat mass (-1.6kg) and increased HDL concentrations (+2mg*dL^{-1}), physical fitness (+1.7mL*min^{-1}*kg^{-1}) and fat oxidation during exercise in both groups (P_{TIME}<0.05), with no between group differences ($P_{TIME*GROUP}$>0.05). HbA1c concentrations significantly decreased after exercise training (P_{TIME}<0.001), with a significant greater reduction in FedEx (-0.30±0.06%) compared to FastEx (-0.08±0.06%; mean difference 0.21%; $P_{TIME*GROUP}$=0.016). No interaction effects were observed for skeletal muscle genes related to lipid metabolism or oxidative capacity.

Conclusion: Endurance-type exercise training in the fasted or fed state do not differ in their efficacy to reduce fat mass, increase fat oxidation capacity, increase cardiorespiratory fitness and HDL concentrations or their risk of hypoglycemia in male patients with type 2 diabetes. Glycated haemoglobin seems to be improved more with exercise performed in the post-prandial compared with the post-absorptive state. **Keywords**: exercise, glycemic control, nutritional status, type 2 diabetes mellitus

Introduction

Exercise or physical activity is considered of key importance in the clinical management of patients with type 2 diabetes mellitus and has, therefore, been included in the guidelines for diabetes prevention and treatment (1). To pursue reasonable and proper glycemic targets (*e.g.* glycated haemoglobin ($HbA1_c$) <6.5% (48 mmol/mol)), current practice guidelines recommend a structured exercise intervention with at least 150 min of moderate-to-vigorous intense aerobic exercise per week, spread over 3-5 days per week, ideally combined with resistance-type exercise (2). The effectiveness of structured aerobic exercise training has clearly been recognized with respect to the improvements in cardiometabolic risk profile, including improved glycemic control (lower $HbA1_c$ concentrations) and increased insulin sensitivity, improved cardiorespiratory fitness, reduced (visceral) adiposity, reduced ectopic lipid stores, lower blood pressure and improvements in blood lipid profile (3-6). Furthermore, exercise is also able to improve cardiac (diastolic) function, resulting from various beneficial remodeling processes in the heart of patients with type 2 diabetes (7). Despite these clinically relevant improvements, much of the inconsistency in the responsiveness to exercise training in patients with type 2 diabetes remains unexplained (8), which advocates for further enhancement of the therapeutic benefits of exercise intervention in these patients (9).

For decades, nutrient-exercise interactions have been studied in the field of sport and exercise science. Of interest, the importance of timing of acute exercise relative to the timing of meals has been associated with glycemic control in patients with type 2 diabetes mellitus (10-12). Changes at different tissue levels (*e.g.* skeletal muscle and adipose tissue) can potentially be clinically relevant to the optimization of therapeutic benefits of physical activity in type 2 diabetes (13), as exercise timing relative to meal ingestion is rarely considered in designing exercise training

studies, especially in type 2 diabetes patients. Of interest, the responses to acute exercise in the fed or fasted state in type 2 diabetes revealed that postprandial exercise more consistently led to reductions in glycemia than prior-meal exercise (in particular when exercise was commenced 30-90 min postprandial). More heterogeneous results are reported for blood insulin concentrations, as recently reviewed by Teo *et al.* (14). It is, however, pivotal to examine if these acute effects of a single exercise bout are translatable to long-term exercise training resulting from consecutive exercise bouts, taken into account the possible risks for (post-exercise) hypoglycemia.

Still, as of today, no long-term (*i.e.* $\geq$ 12 weeks) randomized, controlled exercise training interventions with clinical outcome measures and glycemic control as primary focus have reported the impact of the timing of exercise relative to meal ingestion (15). Of interest, persons with, or at increased risk for, cardiometabolic disease, like patients with type 2 diabetes, would be a very relevant population to investigate the long-term effects of exercise training performed in the overnight-fasted state. Therefore, the aim of the current study was to examine the clinical and metabolic effects, safety and tolerability of exercise training, with exercise performed in either the fasted or the fed state (after consuming a breakfast) in an explorative randomized trial in male patients with type 2 diabetes. Based on studies that have examined the impact of a single exercise bout in the fasted vs. fed state in type 2 diabetes patients, we hypothesized that exercise training in the fed state would be more favorable to improve glycemic control.

Methods

Subjects and randomization

A total of 29 male patients with type 2 diabetes on blood glucose-lowering medication were recruited for this explorative randomized trial. Male patients were included based upon clinical

diagnosis or HbA1c ≥ 48mmol/mol (6.5%), aged 40-80 y, sedentary lifestyle (<2 h structured exercise-related activities per week) and of Caucasian ethnicity. Exclusion criteria were: use of exogenous insulin therapy or clopidogrel treatment, self-reported history of revascularization, coronary artery, renal, pulmonary disease and/or orthopedic disease that would interfere with exercise training. Moreover, patients who were involved in an exercise training or caloric restriction program within one year prior to the study were excluded. Patients were randomly assigned, by envelope, to either three months of exercise training with exercise being performed in the fasted state (before standardized breakfast (which followed after training); FastEx) (*n*=15) or in the postprandial state (after standardized breakfast; FedEx) (*n*=14). To standardize breakfast throughout the intervention, FedEx patients defined their breakfast for the training days and maintained this breakfast throughout the entire intervention. Carbohydrate containing beverages were not allowed during this breakfast, of which the energy content and relative nutrient composition was comparable between FastEx and FedEx groups (see Table, Supplemental Digital Content 1, Relative energy content of breakfast meals, http://links.lww.com/MSS/B765). Due to lack of motivation (*n*=2) or medical reasons not related to the intervention (*n*=2), 4 patients withdrew from the study, leaving 12 patients in the FedEx group and 13 patients in the FastEx group (see Figure, Supplemental Digital Content 2, Flowchart and intervention randomization, http://links.lww.com/MSS/B766). Based on the explorative nature of the study, a post-hoc power was calculated to be 97% (effect size 0.82; α error probability 0.05; total sample size 24). The study was performed in accordance with the standards set by the latest revision (2013) of the Declaration of Helsinki and was approved by the local ethical committees (Jessa Hospital and Hasselt University, Hasselt, Belgium). Written

informed consent was given by all participating patients after careful explanation about the nature and risks of the experimental procedures of the study (registration number NTR4711).

Clinical measurements

Anthropometrics (length and body weight) were determined and body composition was assessed using a Dual Energy X-ray Absorptiometry scan (Hologic Series Delphi-A Fan Beam X-ray Bone Densitometer). Cardiorespiratory fitness was determined by a maximal cardiopulmonary exercise test on a cycle ergometer (eBike Basic, General Electric GmbH, Bitz, Germany), thereby assessing peak oxygen uptake capacity (VO_{2peak}) and workload capacity (W_{peak}) using a 1-min work stage protocol (starting workload 40 Watt (W), incremental workload of 20 W). Oxygen uptake (Jaeger Oxycon, Erich Jaeger GmbH, Germany) and heart rate (using 12-lead electrocardiogram) measurements were performed continuously. All patients cycled until volitional exhaustion. The test was ended when patients were no longer able to maintain a cycling frequency of 55 rpm or higher. Peak exercise effort was confirmed when respiratory gas exchange ratio (RER) was $\geq$1.10, in combination with dyspnea, leg and/or general fatigue.

Indirect calorimetry

During a separate submaximal cycling exercise test in the fasted state energy expenditure (EE; kJ/min) (16), substrate oxidation rates (17) and RER were calculated from VO_2 and VCO_2 determined via indirect calorimetry (Jaeger Oxycon, Erich Jaeger GmbH, Germany) during rest (in supine position) and at 20%, 40% and 60% VO_{2peak}, respectively. During this test, HR was monitored continuously using a 12-lead electrocardiogram.

Blood profiles

After an overnight fast, patients arrived at 08:00 AM to obtain a fasting venous blood sample, which was centrifuged at 4°C for 10 min at 1000 *g* and plasma and serum was stored at -80°C until analysis. Blood samples were measured once by the clinical laboratory (Jessa Hospital, Hasselt, Belgium) and were analyzed for glucose, insulin, total cholesterol, HDL-cholesterol, LDL-cholesterol, plasma triacylglycerols (Roche Cobas 8000, Roche Diagnostics International Ltd, Rotkreuz, Switzerland), HbA1c (Menarini HA-8180 HbA1c auto-analyser, Menarini Diagnostics, Diegem, Belgium) and C-reactive protein (Beckman Synchron LX 20 Analyzer®, Beckman Coulter Inc., Diamond Diagnostics, USA). All coefficients of variation for these assays were less than 15% (ranging from 0.95 to 4.22%). Insulin resistance (IR) was assessed via homeostasis assessment of IR (HOMA-IR), calculated as (fasting plasma glucose (mmol/L)*fasting serum insulin (mU/L))/22.5 (18). Post-intervention blood samples were collected at least 72 hours after the last exercise session to exclude potential residual effects of the last acute exercise bout.

Skeletal muscle biopsy

To investigate gene expression profiles, a skeletal muscle biopsy was taken from the *m. vastus lateralis* under local anesthesia using the Bergström technique with suction during fasting (> 10 h) condition. Dietary intake was recorded three days prior to the skeletal muscle biopsy. A second biopsy was collected following the exercise training intervention (separated by at least 72 hours from the last exercise session) in both groups, after having copied the three-day diet diary of the pre-intervention biopsy. Expression of genes involved in lipolysis (*ATGL*: adipose triglyceride lipase; *HSL*: hormone sensitive lipase; *PLIN 2* and *5*: perilipin 2 and 5),

triacylglycerol synthesis (*DGAT 1* and *2*: diacylglycerol O-acyltranferase 1 and 2) and fatty acid transport (*FABP3*: fatty acid binding protein 3; *CD36*: fatty acid translocase) were analyzed. Furthermore, muscle gene expression of *PPAR-α* (peroxisome proliferators activated receptors-α: beta-oxidation and lipid transport), *PGC-1α* (peroxisome proliferator-activated receptor-γ coactivator 1-α: mitochondrial biogenesis) and *CPT1* (carnitine palmitoyl transferase 1b: mitochondrial transport and oxidation fatty acids) were determined by RT-PCR. Expression profiles of genes of interest were normalized relative to the internal reference gene *β-actin*. RNA primer sequences can be found in the Online supplemental Methods (see Table, Supplemental Digital Content 3, Details of mRNA primer sequences, http://links.lww.com/MSS/B767).

Exercise training protocol

All patients participated in a 12-week individually supervised, endurance-type exercise training program (three exercise sessions per week) while being instructed not to change their habitual diet. During each session, 25 minutes of walking (treadmill, Technogym, Zaventem, Belgium) and 20 minutes of cycling (Excite Bike, Technogym, Zaventem, Belgium) exercise were performed for a total duration of 45 min at an intensity of 65% of baseline VO_{2peak} (heart rate based (Polar, Oy, Finland)). At the end of each training session, calorie consumption data and Borg scores were obtained. Patients in the FastEx group exercised fasted between 07:30-10:00 AM, followed by breakfast and medication intake within 60 min after exercise. Patients in the FedEx group exercised in the postprandial state between 08:00-10:30 AM, after having consumed a breakfast less than 60 min prior to exercise. Water intake was allowed *ad libitum* during the exercise sessions.

Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). Shapiro-Wilk test indicated normal data distribution for patients' characteristics (with the exception of HbA1c), body composition, physical fitness, indirect calorimetry and intervention characteristics. Skewed data were ln-transformed prior to analysis. Group differences were analyzed using independent samples t-test or an unpaired student's t-test (Mann Whitney U test). Intervention effects in both groups were analyzed with two-way repeated-measures ANOVA (with pre- and post-intervention as conditions). In addition to the aforementioned drop-outs, one patient from the FastEx group dropped out during post-intervention testing and was therefore excluded from pre-post intervention analyses. SPSS 22 for Windows was used for all calculations (IBM Corporation, Armonk, NY,U.S.A.). Statistical significance was set at $P<0.05$ (two-tailed).

Results

Clinical characteristics

Patients' characteristics are presented in Table 1. Before the start of the intervention, patients in both FastEx and FedEx were comparable in terms of age, smoking status, diabetes history, HbA1c, body composition (being overweight or slightly obese) and physical fitness (as represented by relative oxygen uptake (VO_{2peak}) and power output (W_{peak})) ($P > 0.05$ for all variables, respectively). With the exception of two patients on DPP-4 inhibitor treatment (monotherapy), most patients in FastEX were on metformin treatment alone (*n*=5) or in combination with either sulfonylurea (*n*=1), DPP-4 inhibitor (*n*=4) or GLP-1 agonist (*n*=1) treatment. In the FedEx group, all patients were on combination therapy, with the exception of

one patient. The dual therapy comprised metformin plus sulfonylurea (n=4), insulin secretagogues (*n*=2), DPP-4 inhibitor (*n*=3) or GLP-1 agonist (*n*=1) treatment, respectively. One patient in the FedEx group was on tritherapy including metformin, sulfonylurea and GLP-1 agonist treatment. In addition to the diabetes treatment, several patients in both groups used other treatments including blood pressure lowering, anti-platelet, lipid lowering or vasodilating therapy (Table 1).

Safety and tolerability

In both FastEx and FedEx, adherence to the exercise sessions was high (91±1 and 94±1% of sessions performed, respectively). Throughout the intervention period, no hypoglycemic events were reported during the sessions in either of the groups. Borg scores indicated good tolerability and remain stable during the intervention period (P_{TIME} >0.1 and $P_{TIME*GROUP}$ >0.1), with a mean score of 12.0±0.4 in the FedEx and 10.6±0.3 in the FastEx during the last 3 weeks of the intervention period (P_{GROUP}=0.098).

Body composition and physical fitness

The intervention progressively led to an increased energy expenditure during the sessions (P_{TIME} < 0.001), resulting in 1669±121 kJ and 1715±133 kJ per session on average in FastEx and FedEx, respectively (P_{GROUP}=0.902, $P_{GROUP*TIME}$=0.434). This increased energy expenditure was associated with an improved body composition, as shown by significant reductions in body weight (P_{TIME}=0.020), body fat percentage (P_{TIME}=0.001) and body fat mass (P_{TIME}<0.001) while preserving fat-free mass (P_{TIME}=0.808) in both FastEx and FedEx (P_{GROUP} and $P_{TIME*GROUP}$ >0.1 for all variables). Fat mass significantly decreased only at the gynoid level (P_{TIME}=0.009), to the same extent in both groups ($P_{TIME*GROUP}$ >0.1). Physical fitness ($VO_{2PEAK/FFM}$ and $W_{peak/FFM}$)

improved significantly following the 12-week intervention (P_{TIME}<0.05) in both FastEx and FedEx (P_{GROUP} and $P_{TIME*GROUP}$ >0.1) (Table 2).

Metabolic blood profile

The exercise intervention was associated with decreased blood HbA1c concentrations in both groups (P_{TIME}<0.001) whereby the reduction was the highest in the FedEx group compared to the FastEx group ($P_{TIME*GROUP}$ =0.016). Fasting plasma glucose, serum insulin concentrations and corresponding HOMA-IR values did not change following the intervention (P_{TIME} and $P_{TIME*GROUP}$ >0.1, respectively) (Figure 2). Regarding lipid metabolism, the intervention was associated with increased plasma HDL-cholesterol concentrations (P_{TIME}=0.019), similarly ($P_{TIME*GROUP}$=0.524) in both FedEx and FastEx. Total cholesterol, LDL-cholesterol and triglyceride concentrations remained unchanged in both groups following the intervention (P_{TIME} and $P_{TIME*GROUP}$ >0.05, respectively). The exercise intervention was not associated with changes in plasma C-reactive protein concentrations (P_{TIME} and $P_{TIME*GROUP}$ >0.1) (Table 2). Individual responses for all blood parameters in both groups are shown in Figure 2.

Substrate oxidation and energy expenditure

The exercise intervention was associated with a significantly increased resting EE in both FedEx and FastEx (P_{TIME}=0.024, $P_{TIME*GROUP}$=0.623; see Figure, Supplemental Digital Content 4, Energy expenditure at rest and during submaximal exercise bouts before (white bars) and after intervention (black bars) in the fed (A) or fasted (B) state, http://links.lww.com/MSS/B768) without any changes in substrate oxidation rates (Table 3). During the submaximal exercise test in the fasted state, EE did not change following the intervention (P_{TIME}>0.1 and $P_{TIME*GROUP}$>0.1, respectively) (see Figure, Supplemental Digital Content 4, Energy expenditure at rest and during

submaximal exercise bouts before (white bars) and after intervention (black bars) in the fed (A) or fasted (B) state, http://links.lww.com/MSS/B768). However, exercise training either performed in the fed or fasted state was associated with a switch in fasting substrate oxidation during the different submaximal cycling intensities, whereby carbohydrate oxidation rates decreased (P_{TIME}<0.05 for the bouts of lowest (20% VO_{2peak}) and highest (60% VO_{2peak}) intensity) and fat oxidation rates increased similarly in both groups (P_{TIME}<0.05 for all three intensities) (Table 3).

Skeletal muscle mRNA expression

Gene expression profiles of fasting skeletal muscle samples are shown in Figure 1. Following intervention, a significant time effect was found only for *PGC1-α* and *IRS-1* mRNA expression (P_{TIME}=0.017 and 0.035, respectively), without any interaction effect. Of interest, *CPT1-β, PLIN5*, *PLIN2* and *GLUT4* mRNA expression tended to be reduced over time (P_{TIME}<0.10). Gene expression of other genes displayed in Figure 1 were not significantly different.

Discussion

In the present study we observed that the benefits of prolonged endurance-type exercise training on body composition, exercise performance, and cardiometabolic risk factors did not differ when exercise was performed in either a post-absorptive or a post-prandial state in male patients with type 2 diabetes. However, HbA_{1C} improved to a greater extent when exercise was performed in the fed as opposed to the fasted state.

The role of feeding status on different aspects of glycemic control is currently under intense but relevant debate, especially in terms of optimizing prevention and treatment strategies for metabolic diseases (15). Acute exercise of moderate intensity in the fed state has been described to more consistently ameliorate glucose concentrations compared to fasted-state exercise in patients with type 2 diabetes (10, 19, 20). Of interest, the most optimal timing to perform exercise was suggested to be mid-postprandial (*i.e.* 30-90 min postmeal) (10, 14). In the present study, the FedEx group performed their exercise sessions within this timeframe, during which hepatic gluconeogenesis and free fatty acids release are suppressed, thereby draining meal-derived glucose from the blood using moderate-intense aerobic exercise. In agreement, the FedEx group showed greater reductions in blood HbA_{1C} content when compared to the FastEx group. However, we did not observe a greater decline in fasting blood glucose concentrations in the FedEx compared with the FastEx group. These results also seem to corroborate recent meta-analyses reporting exercise-induced improvements in long-term glycemic control (HbA_{1C}) (21, 22) without changes in fasting blood glucose concentrations (23) in individuals with type 2 diabetes. Evaluating glycemic control exclusively based on HbA_{1C} would be reductive as it does not imply glycemic variability or the frequency of hypoglycemic events in daily life, two important aspects related to glycemic control which could be different among patients with comparable HbA_{1C} or fasting glucose concentrations (1). Of interest, nutritional behavior (especially carbohydrate consumption) may also be persuasive in affecting HbA_{1C} concentrations (24) and thus may mimic true improvements in glycemic control. However, as the current study lacks standardized tests (*e.g.* an oral glucose tolerance test or continuous glucose monitoring) or wide-ranging diet logs, we could not interpret these facets. Nevertheless, the importance of lowering HbA_{1C} is pivotal since it is linked to mortality (25) and diabetes complications (26),

apart from its link with postprandial hyperglycemia, which might be even more important in the management of diabetes patients with HbA_{1C} <7.5-8 % (27). Accordingly, pre-exercise nutritional state should be considered in future exercise intervention studies as exercise performed in the post-prandial state may result in an overall more effective improvement in glycemic control. However, individual standardization of pre-exercise nutritional status will not reduce variability in dietary habits in the general patient population and thus one should search for other approaches in this regard (*e.g.* practical guidelines on when to perform physical activity or exercise with respect to food intake or choice). Interestingly, improvements in glycemic control following prolonged endurance-type training are also related to the efficacy of an exercise training program to improve body composition (*i.e.* lower body fat mass and augment fat-free mass).

One of the myths in weight loss therapies is the belief that exercise performed in the overnight-fasted state will result in greater fat mass loss when implemented in an exercise training program. Both groups showed a significant reduction in whole-body fat mass (mean fat mass loss of 1.54±0.26 kg), with most fat lost in the gynoid region and no measurable loss of fat-free mass. However, no differences were observed in the amount of fat mass loss between the FedEx and FastEx groups. These findings in type 2 diabetes patients seem to be in line with a recent systematic review postulating no additional benefit of exercise training in the fasted state with respect to body composition changes or fat mass loss, in healthy young individuals and overweight/obese sedentary women following short-term (4-6 weeks) interventions (28). However, focusing solely on fat mass loss does not take physiological adaptations into account which take place with altered body weight and, particularly, altered body composition (29). In this regard, energy balance is a main determinant of weight loss as it considers both food intake

and energy expenditure. Here, energy expenditure (see Figure, Supplemental Digital Content 4, Energy expenditure at rest and during submaximal exercise bouts before (white bars) and after intervention (black bars) in the fed (A) or fasted (B) state, http://links.lww.com/MSS/B768) increased in both groups, presumably due to the sustained or slightly increased lean tissue mass in both groups. Additionally, substrate oxidation rates shifted equally to more fat oxidation capacity (in the fasted state) following the interventions in both groups. Together with the sustained whole body energy expenditure, this may result in a similar negative energy balance between groups, considering no or little compensation of energy intake to every acute exercise bout (15). With respect to weight maintenance or weight loss, however, investigating 24 hour energy intake following fasted and fed state exercise would have been more clinically relevant. Unfortunately, one of the main limitations in the current study was the lack of post-exercise nutritional behavior assessment.

The lack of greater fat mass loss following exercise performed in the fasted versus fed state in the present study may also be, at least partly, explained by the insulin resistant state and impairments in adipose tissue lipid mobilization typically observed in individuals with obesity and type 2 diabetes (30). The relative high blood insulin concentrations chronically present in insulin resistant individuals with type 2 diabetes are responsible for the anti-lipolytic state of the adipose tissue (31), restricting fat mass loss. Besides body fat mass, the effect on blood lipid profile was investigated in the current study. In line with previous work (32), we observed a significant improvement in HDL-cholesterol following the endurance-type training intervention, which did not alter other lipid levels. Despite there were no specific expectations of divergent benefits of exercise performed in the fasted or fed state on lipid profiles, we did look for specific differences between groups. The applied exercise intervention was not associated with changes

in other lipid profile variables, nor for CRP concentrations. Despite the observed body fat mass loss did not translate into improvements in blood lipid profiles, regular aerobic exercise (as prescribed by the current guidelines (1)) is beneficial for ectopic (*e.g.* visceral, liver) fat mass reduction, irrespective of clinically relevant fat mass loss (5).

In addition to a disturbed adipose tissue lipid mobilization, the observed similarity in fat mass loss between groups may partly be explained by a parallel shift in substrate oxidation and an increase in fat oxidation capacity during low-to-moderate intense exercise (20-60% VO_{2PEAK}), as stated earlier. Individuals with type 2 diabetes are known to demonstrate lower lipid oxidation rates per fat-free mass (33). Cross-sectional studies indicated a decreased (34) or similar (35, 36) total lipid oxidation during moderate-intense exercise in type 2 diabetes patients compared to BMI-matched individuals, yet relying less on plasma-derived fatty acids and more on intramyocellular and/or very-low-density lipoprotein lipids (34). The presence of different degrees of insulin sensitivity (37) and the variability in metformin dose (38) may confound these findings and complicate the relationship between lipid oxidative capacity and type 2 diabetes presence in metabolic diseases. Based on the collection of muscle biopsies, only muscle mRNA expression for *PGC1-α* and *PLIN-2* reduced significantly as result of exercise intervention in the FedEx group. These data may suggest that post-transcriptional pathways may have become more efficient, since significant chronic adaptations in clinical outcome measures were noticed within the same timeframe (*e.g.* body composition, fat oxidation, exercise tolerance).

One of the fears when performing exercise in the fasted state in patients with type 2 diabetes is the risk for hypoglycemic events, especially in those treated with long acting sulfonylureas (39). In general and resulting from compensatory endocrine changes (involving insulin, glucagon and catecholamines), stable or slightly elevated blood glucose concentrations are often reported

during acute (≤ 60 min duration) endurance-type exercise performed in the fasted state in healthy individuals (13). In the present study, we did not notice any hypoglycemic symptoms (based on personal communications with the participants or subjective observations by the supervisors) before or following the exercise sessions in either FastEx or FedEx for a total of 804 exercise sessions, which is in line with previous studies (39, 40). Endurance-type exercise in the postprandial state, as applied in this study (mid-postprandial), is suggested to minimize post-exercise hypoglycemic events in patients with type 2 diabetes (10). Based on our findings, both exercise training approaches are well tolerated (based on Borg scale results) and safe to perform, although caution should be taken to interventions of different intensities, durations or modalities (22).

The relative small sample size and inclusion of patients with type 2 diabetes, who were treated with blood glucose lowering medication only, restricts the generalization of our findings to the entire type 2 diabetes patient population. Superior benefits in terms of glycemic control may be achieved when a combined training regimen, including endurance as well as resistance-type exercise, would have been applied (1). Furthermore, data with respect to physical activity levels (outside of the intervention) were not reported in the current study and detailed data regarding post-exercise eating behavior lack, which makes it difficult to firmly draw conclusions about long-term effects of breakfast timing relative to exercise in this population. With respect to nutritional status and exercise in metabolic diseases, it would be valuable to gain more insight in the tissue specific mechanisms taking place, in for example adipose tissue or skeletal muscle, for which functional protein expression data are fundamental.

In conclusion, prolonged endurance-type exercise training in the fasted or fed state are both safe and effective. The applied exercise intervention was associated with reduced fat mass, improved

exercise performance, and increased HDL concentrations in male patients with type 2 diabetes, irrespective of pre-exercise nutritional status. Glycated haemoglobin seems to be improved more with exercise performed in the post-prandial state, although daily glycemic variability and hypoglycemic events should be considered in future research to validate clinical meaningfulness in this population. Yet, healthcare professionals should consider the timing of exercise and food intake in defining individual goals for patients with chronic metabolic disease.

Acknowledgements & Conflict of Interest

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Figure legends

Figure 1 - Relative skeletal muscle gene expression profiles before (black and grey bars for FastEx and FedEx group, respectively) and after intervention (white and squared bars for FastEx and FedEx group, respectively) in the fed or fasted state. Data represent means ± SEM, expressed in arbitrary units; *n*=9 in FastEx and *n*=10 FedEx. Data were normalized to beta-actin and baseline (*i.e*. pre-intervention values (black and grey bars respectively)). * P<0.05 between pre- and post-intervention by paired *t*-tests (when Time effect was observed or showed a tendency). No intervention effects were observed between groups.

Figure 2 – Individual responses of HbA1c, fasting plasma glucose, serum insulin and CRP concentrations (upper panel) and total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides concentrations (lower panel) before and after exercise training in the fed or fasted state. ** P < 0.01 for total group.

Figure 1

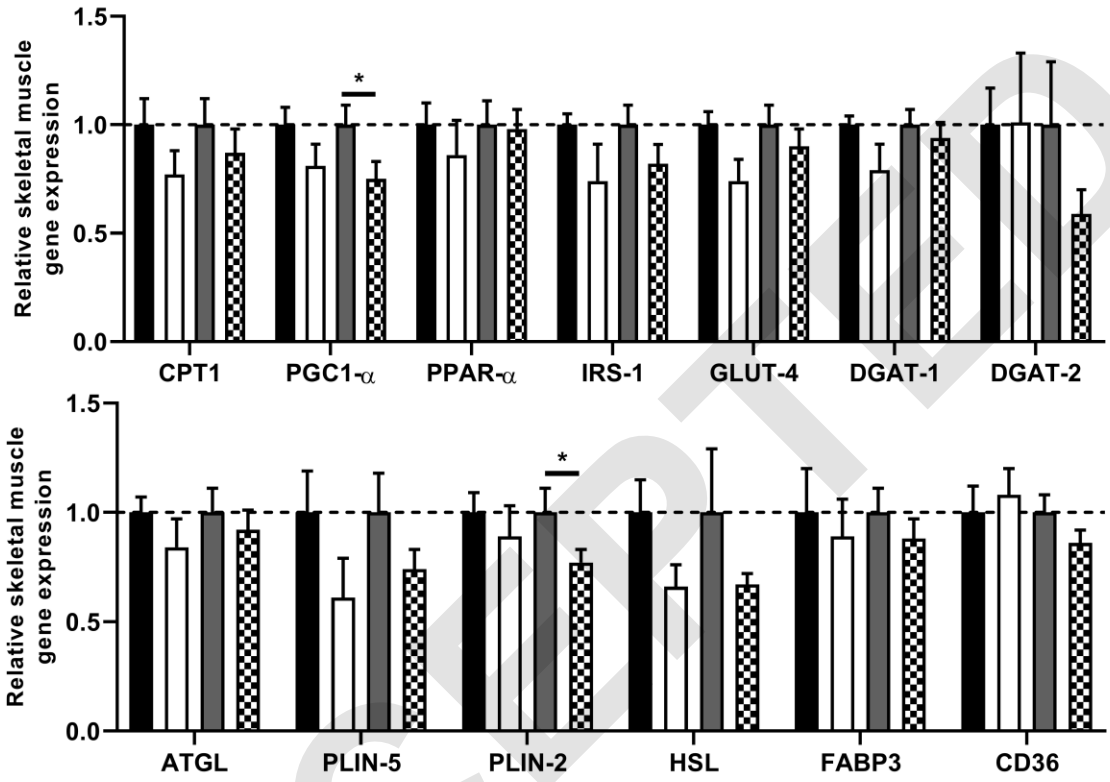


Figure 2

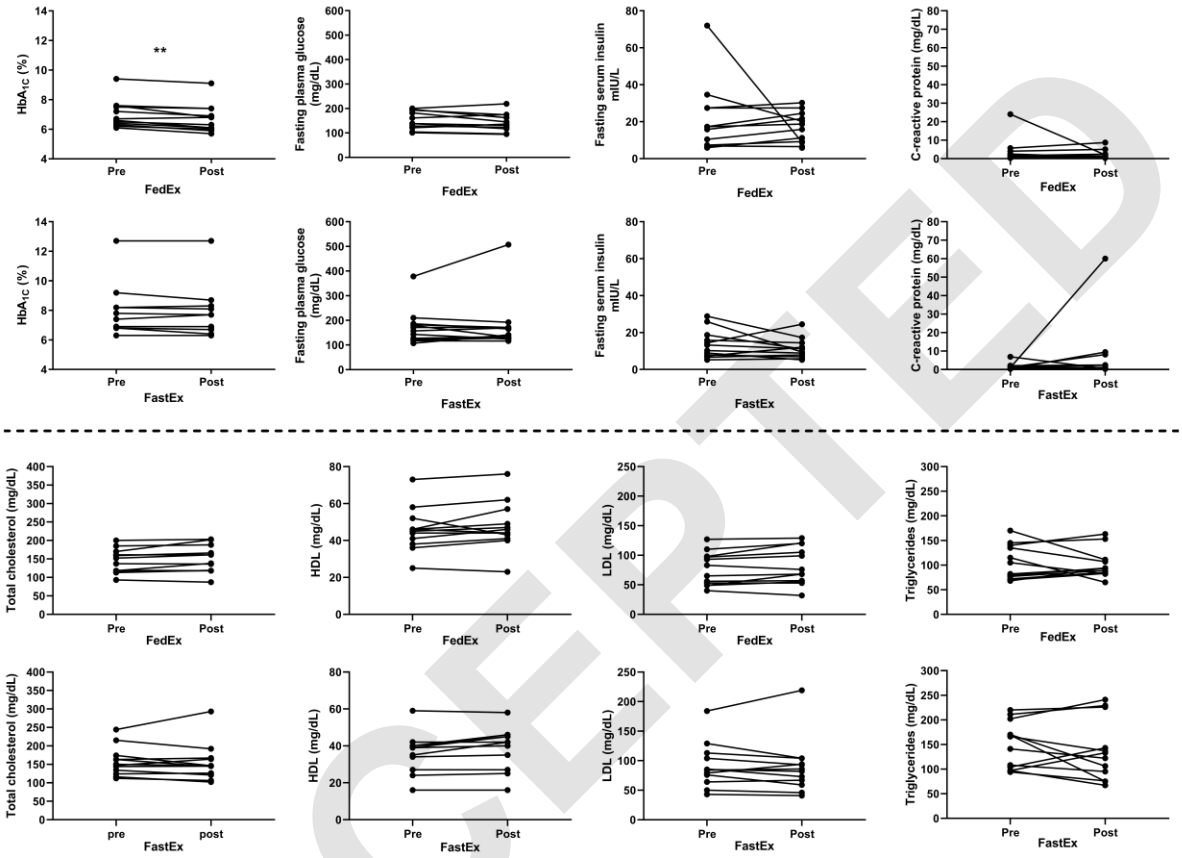


Table 1 Patients' characteristics

Variable	FedEx	FastEx
n	12	13
Age, y	62 ± 1	60 ± 3
Years since diagnosis, y	11.6 ± 1.2	8.0 ± 1.9
Smoking	3/12	2/13
HbA1c, mmol•mol^{-1}	53 ± 2	63 ± 5
HbA1c, %	7.0 ± 0.2	7.9 ± 0.4
Length, cm	175.9 ± 2.0	177.0 ± 1.9
Body weight, kg	94.0 ± 4.5	89.1 ± 3.5
BMI, kg•m^{-2}	30.3 ± 1.3	28.3 ± 0.8
Metformin (*n*)	11	11
Sulfonurea (*n*)	5	1
DPP-4 inhibitor (*n*)	3	6
Insulin secretagogue (*n*)	2	-
GLP-1 agonist (*n*)	2	1
Cardiovascular disease drugs (%)	75	46
Lipid lowering drugs (%)	50	62
Vasodilating drugs (%)	8	-
Other drugs (%)	67	15

Data are mean ± SE.

Table 2 Clinical and metabolic effects of a 12-week exercise training intervention in the fed or fasted state

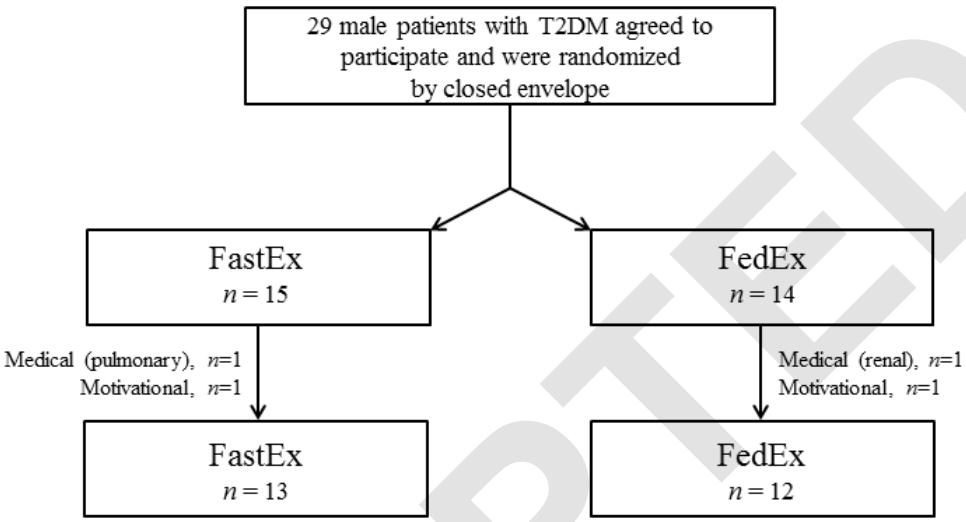
	FedEX (*n*=12)		FastEx (*n*=12)				
	Pre	Post	Pre	Post	P Time	P Group	P Time*Group
Body composition							
Body weight, kg	94.0 ± 4.5	93.5 ± 4.5	90.7 ± 3.3	90.0 ± 3.4	**0.020**	0.554	0.641
BMI, kg/m²	30.3 ± 1.3	30.1 ± 1.3	28.8 ± 0.7	28.5 ± 0.7	**0.023**	0.309	0.575
Body fat, %	33.3 ± 1.5	31.9 ± 1.8	32.0 ± 1.2	30.8 ± 1.3	**0.001**	0.565	0.829
Body fat mass, kg	30.2 ± 2.6	28.5 ± 2.6	27.5 ± 1.7	26.0 ± 1.8	**<0.001**	0.425	0.785
Android fat, kg	3.0 ± 0.3	3.0 ± 0.3	3.0 ± 0.2	3.0 ± 0.2	0.261	0.989	0.547
Gynoid fat, kg	3.8 ± 0.2	3.7 ± 0.2	3.5 ± 0.2	3.4 ± 0.2	**0.009**	0.515	0.621
Fat free mass, kg	58.7 ± 2.0	58.7 ± 2.0	58.0 ± 2.0	57.8 ± 2.0	0.808	0.771	0.711
Physical fitness							
VO2 peak, ml*min-1	2296 ± 199	2314 ± 188	2229 ± 153	2415 ± 185	**0.039**	0.935	0.112
VO2 peak, ml*min-1*kg-1 (FFM)	39.5 ± 3.7	39.7 ± 3.2	38.8 ± 2.8	41.8 ± 2.9	**0.047**	0.876	0.101
Wmax, Watt*kg-1 (FFM)	2.9 ± 0.2	3.1 ± 0.2	2.8 ± 0.1	3.1 ± 0.2	**0.008**	0.909	0.468
RER peak	1.15 ± 0.01	1.20 ± 0.02	1.19 ± 0.02	1.22 ± 0.02	**0.026**	0.421	0.611
HR peak, bpm	144 ± 6	140 ± 6	153 ± 5	152 ± 6	0.136	0.168	0.963
Blood profile							
HbA1c, %	6.6 [6.3 - 7.5]	6.3 [6.0 - 6.9]	7.4 [6.8 - 8.2]	7.7 [6.7 - 8.3]	**<0.001**	0.079	**0.016**
Total cholesterol, mg*dl-1	137 [115 - 170]	138 [118 - 188]	147 [124 - 163]	145 [121 - 164]	0.874	0.597	0.067
LDL cholesterol, mg*dl-1	65 [49 - 98]	68 [55 - 120]	84 [64 - 104]	82 [59 - 94]	0.914	0.488	0.08
HDL cholesterol, mg*dl-1	45 [38 - 52]	46 [41 - 57]	39 [27 - 40]	40 [27 - 45]	**0.019**	0.067	0.524
Triglycerides, mg*dl-1	105 [72 - 140]	94 [83 - 111]	141 [97 - 170]	122 [76 - 143]	0.307	**0.029**	0.417
C-reactive protein, mg*dl-1	1.6 [0.6 - 2.5]	0.9 [0.5 - 2.0]	1.2 [0.5 - 2.1]	1.5 [0.5 - 8.1]	0.879	0.966	0.160
Glucose, mg*dl-1	138 [104 - 193]	131 [96 - 162]	171 [121 - 186]	165 [132 - 171]	0.875	0.241	0.311
Insulin, mIU*L-1	17.3 [7.3 - 27.4]	18.7 [9.2 - 24.5]	13.2 [7.3 - 18.7]	11.1 [7.9 - 14.4]	0.294	**0.044**	0.748
HOMA-IR	7.7 [1.8 - 9.3]	7.9 [2.8 - 8.6]	4.5 [2.8 - 8.5]	4.0 [3.1 - 6.5]	0.181	0.163	0.667

Data are mean ± SE. For blood profiles data are median [interquartile range (IQR)]. FFM: fat free mass; RER: respiratory exchange ratio; VO2 peak: maximum oxygen uptake; Wmax: maximum power output; LDL: low-density lipoprotein; HDL: high-density lipoprotein.

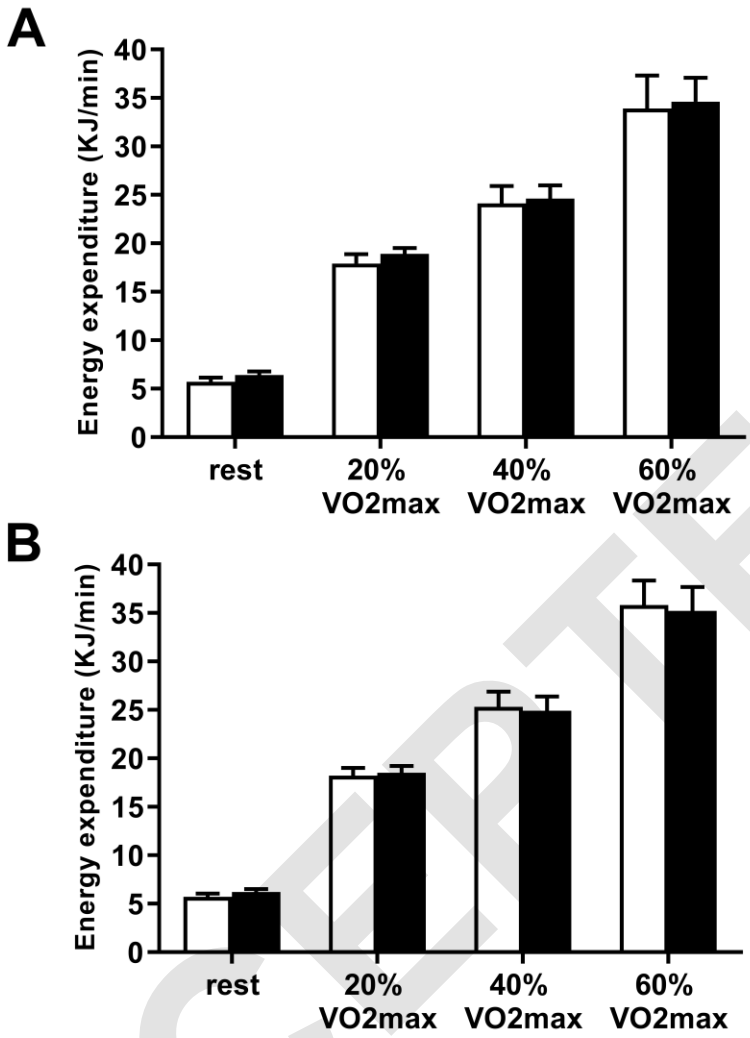
Table 3 Substrate oxidation rates during submaximal exercise before and after exercise training

	FedEx (*n*=12)		FastEx (*n*=11)				
	Pre	Post	Pre	Post	P Time	P Group	P Time*Group
Carbohydrate oxidation (g/min)							
At rest	0.21 ± 0.05	0.23 ± 0.02	0.21 ± 0.03	0.29 ± 0.04†	0.123	0.599	0.294
At 20% VO2 peak	0.49 ± 0.07	0.38 ± 0.05	0.54 ± 0.07	0.44 ± 0.09	**0.042**	0.530	0.888
At 40% VO2 peak	1.10 ± 0.09	0.86 ± 0.11	1.18 ± 0.15	1.04 ± 0.12	0.083	0.375	0.638
At 60% VO2 peak	2.16 ± 0.29	1.73 ± 0.22	2.42 ± 0.30	2.02 ± 0.24	**0.026**	0.429	0.909
Fat oxidation (g/min)							
At rest	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.01 ± 0.01*	0.557	0.385	0.163
At 20% VO2 peak	0.16 ± 0.03	0.23 ± 0.02	0.15 ± 0.02	0.20 ± 0.03*	**0.015**	0.456	0.684
At 40% VO2 peak	0.05 ± 0.02	0.15 ± 0.03*	0.04 ± 0.04	0.09 ± 0.04	**0.024**	0.407	0.371
At 60% VO2 peak	-0.16 ± 0.06	0.02 ± 0.04*	-0.22 ± 0.08	-0.08 ± 0.07	**0.005**	0.327	0.708

Data are mean ± SE. Substrate oxidation rates during submaximal cycling bouts at three different intensities. No differences between groups were observed (P Time*Group > 0.1). VO2 peak: maximum oxygen uptake. * Significantly different from pre-intervention values (*P<0.05; †P<0.01).



Supplemental Figure 1 – Flowchart and intervention randomization



Supplemental Figure 2 - Energy expenditure at rest and during submaximal exercise bouts before (white bars) and after intervention (black bars) in the fed (A) or fasted (B) state. Data represent means ± SEM, *n*=12 in FedEx and FastEx. Overall resting energy expenditure increased following the intervention (P_{TIME}=0.024) with no differences between groups (P_{GROUP}=0.812, $P_{TIME*GROUP}$=0.623).

Supplemental Table 1 Relative energy content of breakfast meals

	FedEX (*n*=10)	FastEx (*n*=10)
Total E (kJ)	1569 ± 301	2004 ± 305
Carbohydrate (% of total E)	60 ± 4	58 ± 4
Protein (% of total E)	16 ± 1	17 ± 2
Fat (% of total E)	23 ± 3	24 ± 4

Data are mean ± SE. Energy content of breakfast was based on the mean of three meals (data from 10 patients in FedEx and 10 patients in FastEx). No differences between groups (P>0.1).

Supplemental Table 2 – Details of mRNA primer sequences

Gene	Gene description	Primer sequence
DGAT1	Diacylglycerol O-Acyltransferase 1	Fw – TAT TGC GGC CAA TGT CTT TGC Re - CAC TGG AGT GAT AGA CTC AAC CA
DGAT2	Diacylglycerol O-Acyltransferase 2	Fw – GAA TGG GAG TGG CAA TGC TAT Re - CCT CGA AGA TCA CCT GCT TGT
IRS1	Insulin receptor substrate 1	Fw – CCC AGG ACC CGC ATT CAA A Re - GGC GGT AGA TAC CAA TCA GGT
PPARα	Peroxisome proliferator-activated receptor alpha	Fw – TCT GAG TCT GTA TGG AGT GAC AT Re - CCA AGT CGT TCA CAT CTA GTT CA
PPARδ	Peroxisome proliferator-activated receptor delta	Fw – AGA AGA ACC GCA ACA AGT GC Re - CTC CCC TCG TTT GCA GTC AG
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha	Fw – AAA AGC CTA AGG AAA CCG TTC TG Re - TAT CGT CCG GGT GGT TGC T
CPT1β	Carnitine palmitoyltransferase 1B	Fw – CGG GAC AGG GGT AAG TTC TG Re - TCT CGC AGG TCT GCT TTT GTG
PLIN5	Perilipin 5	Fw – AAG GCC CTG AAG TGG GTT C Re - GCA TGT GGT CTA TCA GCT CCA
PLIN2	Perilipin 2	Fw – TTG CAG TTG CCA ATA CCT ATG C Re - CCA GTC ACA GTA GTC GTC ACA
HSL	Hormone sensitive lipase	Fw – GCG GAT CAC ACA GAA CCT GGA C Re - AGC AGG CGG CTT ACC CTC AC
ATGL	Adipose triglyceride lipase	Fw – ACC AGC ATC CAG TTC AAC CT Re - ATC CCT GCT TGC ACA TCT CT
FABP3	Fatty acid binding protein 3	Fw – CAT GAC CAA GCC TAC CAC AAT Re - CCC CAA CTT AAA GCT GAT CTC TG
CD36	Fatty acid transporter	Fw – CGA AGT GAT GAT GAA CAG CAG C Re - GAG ACT GTG TTG TCC TCA GCG
GLUT-4	Glucose transporter type 4	Fw – TCT CCA ACT GGA CGA GCA AC Re - CAG CAG GAG GAC CGC AAA TA